

CLAIMS

What is claimed is:

1. A method for identifying a risk for an arthritic disorder in a vertebrate subject, comprising:

comparing the level of at least one indicator of altered mitochondrial function in a biological sample from the subject with a control sample, wherein an altered level of said indicator indicates that the subject has or is at risk for developing an arthritic disorder; and therefrom identifying the risk for the arthritic disorder.

2. A method for determining a degree of disease progression in a vertebrate subject having an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in each of first and second biological samples, said first and second biological samples being obtained from said subject at a first time point and a second time point, respectively, wherein an altered level of said indicator between said first and second time points indicates progression of disease; and therefrom determining the degree of progression of the arthritic disorder.

3. A method of identifying an agent suitable for treating a vertebrate subject suspected of being at risk for having an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in one or more biological samples obtained from the subject in the presence and absence of a candidate agent, wherein an altered level of said indicator indicates that the agent alters mitochondrial function; and therefrom determining the suitability of said candidate agent for treating the arthritic disorder.

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4. A method of determining the suitability of an agent for treating a vertebrate subject suspected of being at risk for having an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in a biological sample obtained from the subject before and after administering to said subject a candidate agent, wherein an altered level of said indicator indicates that the agent alters mitochondrial function; and therefrom determining the suitability of said candidate agent for treating the arthritic disorder.

5. A method of determining the suitability of an agent for treating a vertebrate subject suspected of being at risk for having an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in at least one biological sample obtained from a plurality of subjects before and after administering to each of said subjects a candidate agent, wherein an altered level of said indicator indicates that the agent alters mitochondrial function; and therefrom determining the suitability of said candidate agent for treating the arthritic disorder.

6. A method of stratifying subjects of a vertebrate species according to subtypes of an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in at least one biological sample obtained from each of a plurality of subjects of the species; and therefrom stratifying said subjects according to subtypes of the arthritic disorder.

7. A method of stratifying subjects of a vertebrate species according to subtypes of an arthritic disorder, comprising:

comparing the level of at least one indicator of altered mitochondrial function in a biological sample obtained from each of a plurality of subjects of the species before and after administering to each of said subjects a candidate agent, wherein an altered

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14. The method of claim 13 wherein the nucleated peripheral blood cell is selected from the group consisting of a lymphocyte, a monocyte and a granulocyte.

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15. The method of any one of claims 1-7 wherein the biological sample comprises an articular chondrocyte and the step of comparing comprises comparing the level of at least one indicator of altered mitochondrial function in the absence and presence of transforming growth factor-beta.

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16. The method of claim 15 wherein the indicator of altered mitochondrial activity is nucleotide pyrophosphohydrolase.

10 17. The method of any one of claims 1-7 wherein the biological sample comprises a matrix vesicle derived from a chondrocyte and the indicator of altered mitochondrial function is selected from the group consisting of pyrophosphate, ATP and nucleotide pyrophosphohydrolase.

15 18. The method of any one of claims 1-7 wherein the biological sample comprises a chondrocyte and the indicator of altered mitochondrial function is elaboration by the chondrocyte of at least one extracellular matrix component.

20 19. The method of claim 18 wherein the step of comparing comprises comparing the level of at least one extracellular matrix component that is elaborated in the absence and presence of transforming growth factor-beta.

25 20. The method of claim 18 wherein the extracellular matrix component is selected from the group consisting of collagen, proteoglycan, inorganic pyrophosphate, calcium phosphate and calcium pyrophosphate dihydrate (CPPD).

21. The method of claim 20 wherein the collagen is selected from the group consisting of type I collagen, type II collagen, type X collagen and type XI collagen.

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22. The method of any one of claims 1-7 wherein the biological sample comprises a cybrid cell.

23. The method of any one of claims 1-7 wherein the step of comparing comprises comparing the level of at least one indicator of altered mitochondrial function in the absence and presence of at least one inhibitor of mitochondrial function under conditions and for a time sufficient to decrease the activity of at least one electron transport chain enzyme without decreasing glycolysis.

24. The method of claim 23 wherein the inhibitor of mitochondrial function is selected from the group consisting of antimycin, oligomycin, rotenone and cyanide.

25. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a mitochondrial electron transport chain enzyme.

26. The method of claim 25 wherein the step of comparing comprises measuring electron transport chain enzyme catalytic activity.

27. The method of claim 26 wherein the step of measuring comprises determining enzyme activity per mitochondrion in the sample.

28. The method of claim 26 wherein the step of measuring comprises determining enzyme activity per unit of protein in the sample.

29. The method of claim 25 wherein the step of comparing comprises measuring electron transport chain enzyme quantity.

30. The method of claim 29 wherein the step of measuring comprises determining enzyme quantity per mitochondrion in the sample.

31. The method of claim 29 wherein the step of measuring comprises
5 determining enzyme quantity per unit of protein in the sample.

32. The method of claim 25 wherein the mitochondrial electron transport chain enzyme comprises at least one subunit of mitochondrial complex I.

10 33. The method of claim 25 wherein the mitochondrial electron transport chain enzyme comprises at least one subunit of mitochondrial complex II.

34. The method of claim 25 wherein the mitochondrial electron transport chain enzyme comprises at least one subunit of mitochondrial complex III.

15 35. The method of claim 25 wherein the mitochondrial electron transport chain enzyme comprises at least one subunit of mitochondrial complex IV.

20 36. The method of claim 35 wherein the at least one subunit of mitochondrial complex IV is selected from the group consisting of COX1, COX2 and COX4.

25 37. The method of claim 25 wherein the mitochondrial electron transport chain enzyme comprises at least one subunit of mitochondrial complex V.

38. The method of claim 37 wherein the at least one subunit of mitochondrial complex V is selected from the group consisting of ATP synthase subunit 8 and ATP synthase subunit 6.

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39. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is the amount of ATP per cell in the sample.

40. The method of claim 39 wherein the step of comparing comprises
5 measuring the amount of ATP per mitochondrion in the sample.

41. The method of claim 39 wherein the step of comparing comprises measuring the amount of ATP per unit protein in the sample.

10 42. The method of claim 39 wherein the step of comparing comprises measuring the amount of ATP per unit mitochondrial mass in the sample.

43. The method of claim 39 wherein the step of comparing comprises measuring the amount of ATP per unit mitochondrial protein in the sample.

15 44. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is the rate of ATP synthesis in the sample.

20 45. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is an ATP biosynthesis factor.

46. The method of claim 45 wherein the step of comparing comprises measuring ATP biosynthesis factor catalytic activity.

25 47. The method of claim 46 wherein the step of measuring comprises determining ATP biosynthesis factor activity per mitochondrion in the sample.

48. The method of claim 46 wherein the step of measuring comprises determining ATP biosynthesis factor activity per unit mitochondrial mass in the sample.

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49. The method of claim 46 wherein the step of measuring comprises determining ATP biosynthesis factor activity per unit of protein in the sample.

5 50. The method of claim 45 wherein the step of comparing comprises measuring ATP biosynthesis factor quantity.

10 51. The method of claim 50 wherein the step of measuring comprises determining ATP biosynthesis factor quantity per mitochondrion in the sample.

52. The method of claim 50 wherein the step of measuring comprises determining ATP biosynthesis factor quantity per unit of protein in the sample.

15 53. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a mitochondrial matrix component.

54. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a mitochondrial membrane component.

20 55. The method of claim 54 wherein the mitochondrial membrane component is a mitochondrial inner membrane component.

25 56. The method of claim 54 wherein the mitochondrial membrane component is selected from the group consisting of adenine nucleotide translocator (ANT), voltage dependent anion channel (VDAC), malate-aspartate shuttle, calcium uniporter, UCP-1, UCP-2, UCP-3, a hexokinase, a peripheral benzodiazepine receptor, a mitochondrial intermembrane creatine kinase, cyclophilin D, a Bcl-2 gene family encoded polypeptide, tricarboxylate carrier and dicarboxylate carrier.

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57. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a Krebs cycle enzyme.

58. The method of claim 57 wherein the step of comparing comprises
5 measuring Krebs cycle enzyme catalytic activity.

59. The method of claim 58 wherein the step of measuring comprises determining enzyme activity per mitochondrion in the sample.

10 60. The method of claim 58 wherein the step of measuring comprises determining enzyme activity per unit of protein in the sample.

61. The method of claim 57 wherein the step of comparing comprises measuring Krebs cycle enzyme quantity.

15 62. The method of claim 61 wherein the step of measuring comprises determining enzyme quantity per mitochondrion in the sample.

20 63. The method of claim 61 wherein the step of measuring comprises determining enzyme quantity per unit of protein in the sample.

64. The method of claim 57 wherein the Krebs cycle enzyme is citrate synthase.

25 65. The method of claim 57 wherein the Krebs cycle enzyme is selected from the group consisting of aconitase, isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinyl-coenzyme A synthetase, succinate dehydrogenase, fumarase and malate dehydrogenase.

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66. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is mitochondrial mass per cell in the sample.

67. The method of claim 66 wherein mitochondrial mass is determined
5 using a mitochondria selective agent.

68. The method of claim 66 wherein mitochondrial mass is determined using nonylacridine orange.

10 69. The method of claim 66 wherein mitochondrial mass is determined by morphometric analysis.

70. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is the number of mitochondria per cell in the sample.

15 71. The method of claim 70 wherein the step of comparing comprises measuring a mitochondrion selective reagent.

20 72. The method of claim 71 wherein the mitochondrion selective reagent is fluorescent.

73. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a co-predictor of altered mitochondrial function comprising the amount of mitochondrial DNA per cell in the sample and the step of comparing further
25 comprises comparing at least one additional indicator of altered mitochondrial function.

74. The method of claim 73 wherein the step of comparing comprises measuring mitochondrial DNA by contacting a biological sample containing mitochondrial DNA with an oligonucleotide primer having a nucleotide sequence that is complementary

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to a sequence present in said mitochondrial DNA, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

detecting hybridization of the primer to the mitochondrial DNA, and therefrom quantifying the mitochondrial DNA.

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75. The method of claim 74 wherein the step of detecting comprises a technique selected from the group consisting of polymerase chain reaction, oligonucleotide primer extension assay, ligase chain reaction, and restriction fragment length polymorphism analysis.

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76. The method of claim 73 wherein the step of comparing comprises measuring mitochondrial DNA by contacting a sample containing amplified mitochondrial DNA with an oligonucleotide primer having a nucleotide sequence that is complementary to a sequence present in said amplified mitochondrial DNA, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

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detecting hybridization of the primer to the mitochondrial DNA, and therefrom quantifying the mitochondrial DNA.

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77. The method of claim 76 wherein the step of detecting comprises a technique selected from the group consisting of polymerase chain reaction, oligonucleotide primer extension assay, ligase chain reaction, and restriction fragment length polymorphism analysis.

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78. The method of claim 76 wherein the mitochondrial DNA is amplified using a technique selected from the group consisting of polymerase chain reaction, transcriptional amplification systems and self-sustained sequence replication.

79. The method of claim 73 wherein the step of comparing comprises measuring mitochondrial DNA by contacting a biological sample containing mitochondrial

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DNA with an oligonucleotide primer having a nucleotide sequence that is complementary to a sequence present in said mitochondrial DNA, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

detecting hybridization and extension of the primer to the mitochondrial
5 DNA to produce a product, and therefrom quantifying the mitochondrial DNA.

80. The method of claim 73 wherein the step of comparing comprises measuring mitochondrial DNA by contacting a sample containing amplified mitochondrial DNA with an oligonucleotide primer having a nucleotide sequence that is complementary
10 to a sequence present in said amplified mitochondrial DNA, under conditions and for a time sufficient to allow hybridization of said primer to the mitochondrial DNA; and

detecting hybridization and extension of the primer to the mitochondrial DNA to produce a product, and therefrom quantifying the mitochondrial DNA.

15 81. The method of claim 80 wherein the mitochondrial DNA is amplified using a technique selected from the group consisting of polymerase chain reaction, transcriptional amplification systems and self-sustained sequence replication.

20 82. The method of claim 73 wherein the amount of mitochondrial DNA in the sample is determined using an oligonucleotide primer extension assay.

83. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is free radical production.

25 84. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is selected from the group consisting of reactive oxygen species, protein nitrosylation, protein carbonyl modification, DNA oxidation, mtDNA oxidation, protein oxidation, protein carbonyl modification, malondialdehyde adducts of proteins, a glycoxidation product, a lipoxidation product, 8'-OH-guanosine adducts and TBARS.

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85. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is reactive oxygen species.

5 86. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is protein nitrosylation.

87. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is DNA oxidation.

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88. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is mitochondrial DNA oxidation.

15 89. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is protein carbonyl modification.

90. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a cellular response to elevated intracellular calcium.

20 91. The method of any one of claims 1-7 wherein the indicator of altered mitochondrial function is a cellular response to at least one apoptogen.

25 92. A method of treating a human patient having an arthritic disorder, comprising administering to the patient an agent that substantially restores to a normal level at least one indicator of altered mitochondrial function.

93. The method of claim 92 wherein the indicator of altered mitochondrial function is selected from the group consisting of a mitochondrial electron

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transport chain enzyme, a Krebs cycle enzyme, a mitochondrial matrix component, a mitochondrial membrane component and an ATP biosynthesis factor.

94. The method of claim 93 wherein the indicator of altered
5 mitochondrial function is selected from the group consisting of mitochondrial number per cell and mitochondrial mass per cell.

95. The method of claim 93 wherein the indicator of altered
10 mitochondrial function is an ATP biosynthesis factor.

96. The method of claim 92 wherein the indicator of altered
15 mitochondrial function is selected from the group consisting of the amount of ATP per mitochondrion, the amount of ATP per unit mitochondrial mass, the amount of ATP per unit protein and the amount of ATP per unit mitochondrial protein.

97. The method of claim 92 wherein the indicator of altered
mitochondrial function comprises free radical production.

98. The method of claim 92 wherein the indicator of altered
20 mitochondrial function comprises a cellular response to elevated intracellular calcium.

99. The method of claim 92 wherein the at least one indicator of altered
mitochondrial function is a co-predictor of altered mitochondrial function.

100. The method of claim 99 wherein the co-predictor of altered
25 mitochondrial function is an amount of mitochondrial DNA per cell in the patient.

101. A method of treating an arthritic disorder, comprising administering
an effective amount of a mitochondrial function-altering agent.

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102. The method of claim 101 wherein the agent is a mitochondria protective agent.

5 103. The method of claim 101 wherein the agent is an antioxidant.

104. A kit for identifying a risk for, determining a degree of disease progression of, or stratifying subjects of a vertebrate species according to subtypes of, an arthritic disorder in a biological sample from a vertebrate subject, comprising:

10 a) at least one reagent capable of detecting a level of at least one indicator of altered mitochondrial function according to the method of any one of claims 1, 2, 6 or 7; and

b) ancillary reagents suitable for use in detecting said indicator.

15 105. A method of preparing a synthetic cartilage patch, comprising:
introducing heterologous mitochondrial DNA into chondrocytes from a first subject having an arthritic disorder to generate cybrid chondrocytes, wherein said exogenous mitochondrial DNA is from a second subject known not to have an arthritic disorder; and culturing said cybrid chondrocytes under conditions and for a time sufficient
20 to permit said chondrocytes to elaborate extracellular matrix.

106. A method of preparing a synthetic cartilage patch, comprising:
culturing chondrocytes from a subject having an arthritic disorder in the presence of at least one mitochondria protective agent under conditions and for a time
25 sufficient to permit said chondrocytes to elaborate extracellular matrix.

107. The method of claim 106, wherein the chondrocytes are cultured in the presence of at least one mitochondria protective agent that is an antioxidant.

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108. A method of preparing a synthetic cartilage patch, comprising:

selecting a subpopulation of chondrocytes having enhanced mitochondrial function from an unselected population of chondrocytes derived from a subject having an arthritic disorder; and

5 culturing said subpopulation of chondrocytes under conditions and for a time sufficient to permit said chondrocytes to elaborate extracellular matrix.

109. A synthetic cartilage patch produced by the method of any one of claims 105, 106 or 108.

10 110. A method of repairing a cartilage defect at a predetermined site in a subject comprising surgically implanting the synthetic cartilage patch of claim 109 at said predetermined site.

15 111. The method of claim 110 further comprising administering one or more mitochondria protective agents to said subject.

112. The method of claim 111, wherein said one or more mitochondria protective agents comprises at least one antioxidant.

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